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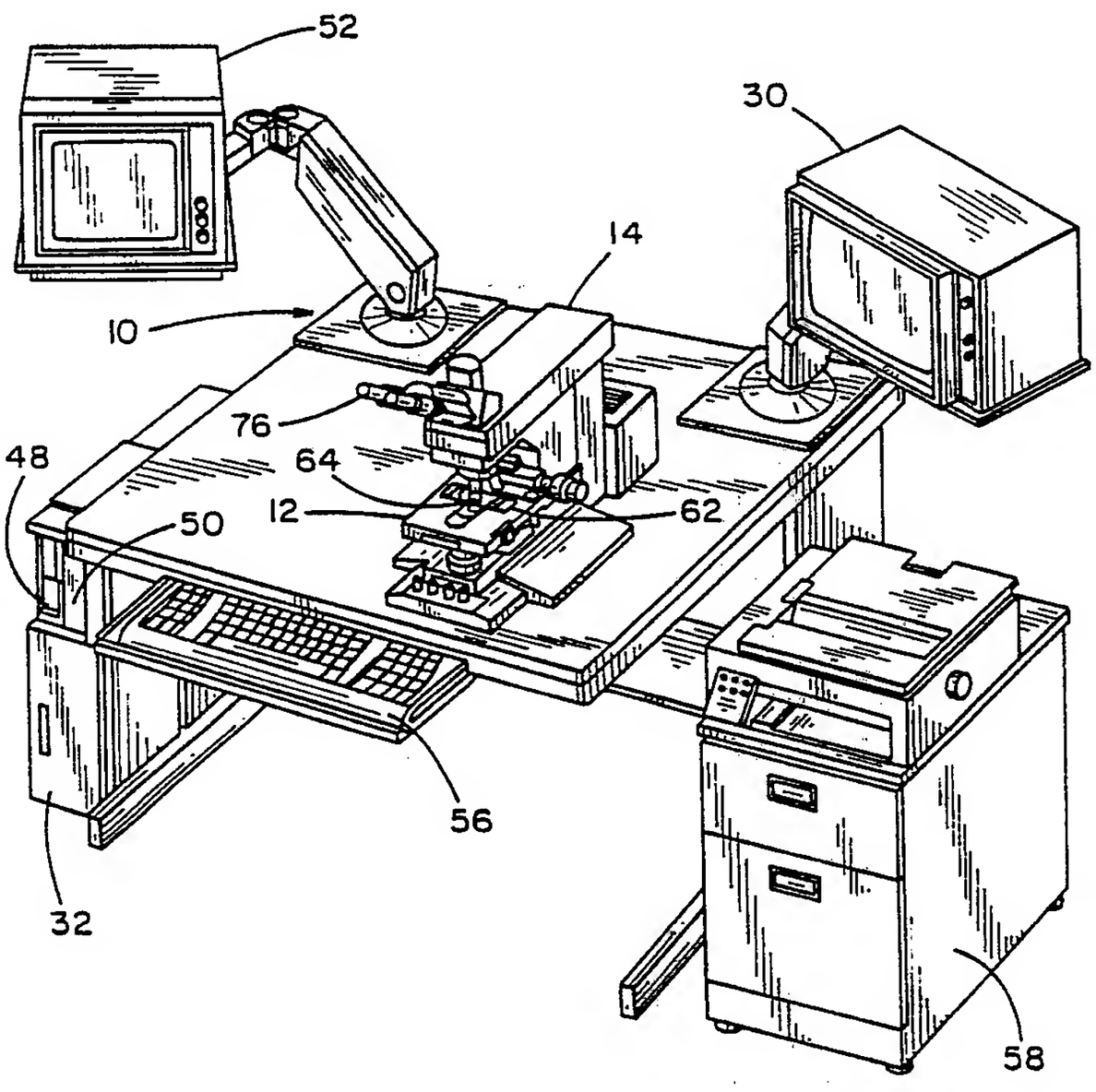
(54) Title: METHOD AND APPARATUS FOR DETERMINING A PROLIFERATION INDEX OF A CELL SAMPLE

(57) Abstract

An image processing method and apparatus (10) determines a proliferation index of a cell sample by staining the cells with a chromogen for a proliferation substance and a counterstain for the cell nuclei. The chromogen is activated by an antibody-enzyme conjugate which binds to the proliferation substance to produce a stained cell sample. The stained cell sample is examined with an optical microscope (12), forming a portion of the apparatus, which produces a magnified cell sample image. The apparatus optically filters (18) the cell sample image and produces a pair of optically enhanced proliferation substance and cell nuclei images. The enhanced images are electronically analyzed to determine the amounts of cell nuclei and proliferation substance appearing in the images, respectively. The amounts are then compared to yield a proliferation index for the portion of the cell sample appearing in the cell sample image.



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METHOD AND APPARATUS FOR DETERMINING A PROLIFERATION INDEX OF A CELL SAMPLE

BACKGROUND OF THE INVENTION

The invention relates to a system for performing a biochemical assay of a cell sample to provide an accurate quantitative analysis of a characteristic of the cells which have been sampled. More particularly, the invention is directed to a system which receives images of stained cells and enhances the cell images prior to further processing to determine the proliferation index of the enhanced cell images.

One of the problems which faces pathologists in their clinical practice is that of determining whether a cell sample taken from a patient during a biopsy procedure or the like is benign or malignant. Although a surgeon may have a good intuition about the type of tissue mass which he has removed, nevertheless he must confirm his preliminary diagnosis with a histological examination of the cell sample removed from the patient. The histological examination entails cell staining procedures which allow the morphological features of the cells to be seen relatively easily in a light microscope. A pathologist after having examined the stained cell sample, makes a qualitative determination of the state of the tissue or the patient from whom the sample was removed and reaches a conclusion as to whether the patient is normal, has a premalignant condition which might place him at risk of a malignancy in the future or has cancer. While this diagnostic method has provided some degree of predictability in the past it is somewhat lacking in scientific rigor since it is heavily reliant on the subjective judgement of the pathologist.

Attempts have been made to automate the cellular examination process. In U.S. Patent No. 4,741,043 to Bacus for Method and Apparatus for Image Analyses of Biological Specimens, an automated method and a system for measuring the DNA of cells are disclosed which employ differential staining of the DNA in cell nuclei with a Feulgen Azure A stain and image processing. While the system provides an accurate assay of the cellular DNA its predictive power for cell replication, a key indicator of the presence of cancer, could be improved.

It is well known that cells follow a replication cycle. Most somatic cells of an adult human replicate at a relatively slow rate, only rapidly enough to replace cells shed by the body and lost to normal cellular wear and tear. At any instant, most of those somatic cells are in the G0 or resting phase of the replication cycle. When they leave the resting phase they enter the G1 or first gap phase but are not yet producing extra DNA. Upon becoming committed to the S-phase, however, they do produce other material such as proliferation substances e.g. cyclin and other S-phase proteins. The cells in the synthesis or S-phase are actively synthesizing DNA and produce double the amount of DNA normally contained in the cell nuclei in preparation for mitosis or division of the cell nuclei during cell replication. A normal human somatic cell contains 23 chromosome pairs and is in the diploid state. The diploid state is also referred to as the 2N state. At the time of replication the number of chromosome pairs increases to 46, double the normal amount in anticipation of cell division. The chromosome state immediately before replication is referred to as the 4N state. The cells then enter the second gap phase or G2 phase in which little or no DNA is synthesized. Following the G2 phase is the mitosis or M-phase in which the cells themselves divide. If the cells are actively proliferating they may reenter the G1 phase.

Although DNA analysis may be adequate for estimating the number or proportion of proliferating cells in normal cells or tissue, it should be appreciated that this is not the case with malignant cells, the very ones for which it often is important to know the extent of proliferation. This is because malignant cells often have increased amounts of DNA, even in the G0 phase, due to the increased chromosome count. Therefore, it is impossible to conclude with certainty from a DNA analysis that a particular cell, e.g. one having 1.5 times the normal DNA content, is a malignant cell with additional chromosomes or chromosome parts or is a normal cell which is halfway through the S-phase having only replicated one-half the DNA necessary for cell division. Thus it is clear that an analysis method independent of DNA, utilizing other markers, such as variously produced proteins associated with S-phase proliferation and the cell division process, has many advantages

It should also be appreciated that quantitating on cellular proliferation indices has previously been performed by counting the numbers of cells in a cell sample carrying an indicator or stain for a proliferation substance. For instance, a well known method of determining the proliferation index is to stain the cells with an immunofluorescent dye which binds to cyclin and manually count the stained cells to determine the proportion of cells having proliferation substance.

Another method of determining the proliferation index of cells is the grain counting method. In that method, tritiated thymidine is added to a cell culture growth medium. Proliferating cells take up the tritiated thymidine and incorporate it into DNA being synthesized in the cells. The cells are then fixed and placed in proximity with a photographic emulsion. Decay products of the tritium expose portions of the emulsion. The exposed portions may be visualized as grains by photographic

development processes. The grains are then counted to determine whether the cells are normal or abnormal. One of the drawbacks of this method lies in the fact that it is very time consuming. It is necessary that the cells be harvested alive and kept alive long enough to take up the tritiated thymidine. The cells must then be fixed and held in proximity with the emulsion in order to expose it. Since relatively low intensities of radiation may emanate from the cells, it may take days or even weeks to obtain a latent image on the emulsion, which must then be developed. In the meantime, the patient's disease may be progressing.

One of the drawbacks of the prior art methods is that they are prone to human error due to the tedium of counting the cells on a microscope slide under high magnification. Often the people examining the slides only are able to estimate the relative number of cells which show a positive result for proliferation substance.

The prior imaging systems have also suffered from the problem that while they usually accurately identify the images of cell objects in an image being processed they do not always accurately identify boundaries of the cell objects being evaluated. This may be a problem when an assay is being performed on the cell objects on the basis of their image areas.

The prior art methods of quantitatively analyzing the cell samples for proliferation substances could not be automated simply. This is because it is necessary to determine a baseline value for the total number of cells examined as opposed to the number of cells which have proliferation substance. In order to make this type of evaluation an automatic system must be able to recognize what constitutes a cell or a cell nucleus. In order to solve this baseline recognition problem the instant invention employs separate stains for the cell nuclei and the proliferation substances. In addition, the stains are

separated spectrally so that they can be readily distinguished by optical filters which are compatible with them. The optical separation of the two components to be measured makes the subsequent analysis of the cell images more convenient to automate.

A similar difficulty is encountered in an image analysis based on cell object areas when cell objects images overlap, touch or otherwise share contiguous areas. In that case, what is actually a double or triple object image may not be tallied properly resulting in an inaccurate result or conclusion.

SUMMARY OF THE INVENTION

The present invention provides a rapid and convenient method and an apparatus for practicing that method for determining the amount of a proliferation substance in a cell sample. The cell sample may be a tissue sample or a cell preparation. Tissue samples are frozen sections or paraffin sections of connected cells. The cell preparations are made from body fluids such as cerebrospinal fluid, blood, pleural effusions and the like. Cell preparations may also be made from needle aspirates of tumors, cysts or lymph nodes. Cell preparations may also be made from touch preparations which are made by touching a freshly microtomed surface of a piece of tissue to a microscope slide to which the cells cling. In particular, the apparatus and method employ a rabbit anti-mouse immunoglobulin (IgG) based staining system wherein antibodies for a proliferation substance such as cyclin or the antigen for Ki-67 are complexed with an enzyme in this embodiment horseradish peroxidase (HRP). The cells are contacted with the HRP-proliferation substance antibody conjugate which binds only to portions of the cells which have epitopes identifying them as proliferation substance. A stain, in this embodiment 3,

3' diaminobenzidine tetrahydrochloride (DAB), and hydrogen peroxide H_2O_2 are placed in contact with the cells having the antibody-HRP conjugate bound to their proliferation substance sites. The HRP catalyzes a chromogen forming reaction only at the areas where it is bound. The catalyzed chromogen forming reaction produces a red-brown chromogen precipitate bound to proliferation sites.

The cells are then stained with a counterstain, in this instance ethyl green, which is commonly known as methyl green. The image of the cells is magnified in a light microscope and split into a pair separated images. The separated images are enhanced by a pair of narrow bandpass optical filters. One of the narrow bandpass optical filters preferentially transmits light having a wavelength at the transmission peak of the counterstain thereby producing an optically enhanced proliferation substance image which only has background and the red-brown chromogen. The background of the proliferation substance image is composed of the cell nuclei, cytoplasm and the like which have substantially zero optical density. The proliferation substance sites have a relatively high optical density. Thus the only features which are easily perceivable are the proliferation substance sites.

The other narrow bandpass optical filter preferentially transmits the red-brown transmission peak and blocks the counterstain peak thereby enhancing optical density differences between the cell nuclei and the proliferation substance chromogen. The filter produces an optically enhanced cell nuclei image which has only background features and the cell nuclei.

The inventive apparatus senses the enhanced proliferation substance image with a first monochrome television camera. The enhanced cell nuclei image is sensed by a second monochrome television camera. Analog signals representative of the images are fed to respective

image processors. The image processors convert the analog signals to digitized arrays of pixels which are stored in internal frame buffers.

When a tissue section is being examined the apparatus computes an area of the proliferation substance image which has high optical density, yielding an area measure for the proliferation substance in that image field. When a cell preparation is being examined the apparatus computes the proliferation index on the basis of the percentage of cell nuclei having more than a threshold amount of proliferation substance therein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an isometric view of an apparatus for determining a proliferation index of a cell sample embodying the present invention;

FIG. 2 is a block diagram of the apparatus of FIG. 1;

FIG. 3 is an elevational view of an optical conversion module of the apparatus of FIG. 1;

FIG. 4 is a magnified view of a stained cell sample as seen through the microscope of FIG. 1 without optical filtering;

FIG. 5 is a magnified view of the stained cell sample of FIG. 4 as seen through a 620 nanometer narrow band optical filter which yields a cell nuclei image;

FIG. 6 is a magnified view of the stained cell sample of FIG. 4 as seen through a 500 nanometer narrow band optical filter which yields a proliferation substance image;

FIG. 7 is a graph of the spectral response of a chromogen, a counterstain and the narrow band optical filters;

FIG. 8 is a flow chart of a sequence of steps performed by the apparatus of FIG. 1 in selecting a cell sample analysis mode;

FIG. 9 is a flow chart of a sequence of steps performed by the apparatus of FIG. 1 in determining the proliferation index of a tissue section cell sample;

FIG. 10 is a flow chart of the steps carried by the apparatus in determining the proliferation index of a cell preparation cell sample;

FIG. 11 is a screen display of the tissue screen; and

FIG. 12 is a screen display of the cell preparation screen.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Referring now to the drawings and especially to FIG. 1, an apparatus embodying the present invention and generally identified by numeral 10 is shown therein. The apparatus 10 comprises an optical microscope 12, which may be of any conventional type but in this embodiment is a Reichart Diastar or Microstar. An optical conversion module 14 is mounted on the microscope 12 to enhance optically a magnified image of a cell sample viewed with the microscope 12. The optical conversion module 14, as may best be seen in FIG. 3, has a cell nuclei sensing means comprising a cell nuclei image optical enhancement unit 16. The cell nuclei image optical enhancement unit 16 has a 620 ± 20 nanometer red narrow bandpass optical transmission filter 18 and a television camera 20 for receiving a filtered image from the filter 18. A proliferation substance sensing means comprising a proliferation substance optical enhancement module 22 has a green 500 ± 20 nanometer narrow bandpass optical transmission filter 24 and a television camera 26 and is also part of the optical conversion module 14. Each of the television cameras 20 and 26 generates a standard NTSC compatible signal representative, respectively, of an enhanced cell nuclei image and an enhanced proliferation substance image. An image processing system 28 is

connected to the television cameras 20 and 26 to receive the enhanced cell nuclei image signal and the enhanced proliferation substance image signal and to store a cell nuclei pixel array and a proliferation substance prixel array therein. The image processor 28 is connected to a computer 32, in the present embodiment, an IBM personal computer model AT for processing of the cell nuclei and proliferation substance pixel arrays.

The computer 32 includes a system bus 34, connected to the image processor unit 28. An 80286 microprocessor 36 is connected to the system bus 34. A random access memory 38 and a read only memory 40 are also connected to the system bus 34 for storage of information. A disk controller 40 is connected by a local bus 44 to a Winchester disk drive 46 and to a floppy disk drive 48 for secondary information storage. A video conversion board 50 in this embodiment, an EGA board having 256K bytes of memory, is connected to the system bus 34 to control an instruction monitor 52 connected to the EGA board 50. A keyboard processor 54 is connected to the system bus 34 to interpret signals from a keyboard 56 which is connected to the keyboard processor 54. A printer 58 is connected to the system bus 54 for communication therewith. An X Y or image field board 60 is connected to the system bus 34. The X Y board 60 also is connected to a slide holder of the microscope 12 to sense the relative position of a slide 62 with respect to a microscope objective 64 and thus identify a field being viewed. Included is a Y position sensor 66 and an X position sensor 68. The Y position sensor 66 is connected via a communication path 70 to the X Y board 60. The X position sensor 68 is connected via a communication path 72 to the X Y board 60. The microscope 12 also includes an eyepiece 76 in optical alignment with the objective 74 for magnification of light forming an image of a cell sample on the slide 62.

The method of the instant invention is practiced by collecting a cell sample, which may be in the form of a tissue section made from a frozen section or a paraffinized section and having both cell nuclei, cell fragments and whole cells therein. Alternatively, the cell sample may be a cell preparation of the type which might be taken from blood, pleural effusions, cerebrospinal fluid, or by aspirating the contents of a cyst or a tumor. The cells of the cell sample are placed on the slide 62 and fixed thereon. A monoclonal antibody for a proliferation substance to be detected in the cells is then placed in contact with them. The monoclonal antibody may for instance be Ki-67 or may be an antibody for, 5-bromodeoxyuridine, for cyclin or for other proteins which indicate that cellular replication is occurring. The monoclonal antibody selectively binds to all points on and within the cells where the proliferation substance is present. The monoclonal antibody also has bound thereto a bridging antibody and a peroxidase anti-peroxidase complex. The anti-peroxidase comprises an antibody which specifically binds to the enzyme peroxidase. The peroxidase enzyme is bound to the antibody and held through the chain of antibodies to the proliferation substance in the cells.

In order to view the proliferation substance sites, a quantity of a mixture containing hydrogen peroxide and 3, 3' diaminobenzidine tetrahydrochloride (DAB) is applied to the cell sample on the slide 62. The hydrogen peroxide and the DAB react to form a chromogen consisting of a reddish-brown precipitate. The usual rate of reaction however is relatively low. The peroxidase catalyzes the chromogen-forming reaction only at the points where the peroxidase is localized. Thus, chromogen is precipitated only at the points in the cells where proliferation substance is present and the cells are preferentially stained only at the points where they

have proliferation substance. After a period of about 15 minutes, the unreacted DAB and hydrogen peroxide are removed from the cell sample. The cells are then counterstained with methyl green (more properly known as ethyl green) which preferentially binds with the cell nuclei. Thus, cell nuclei are stained and the points within the cell nuclei having proliferation substance are stained reddish-brown.

The microscope slide 62 is then placed on a carrying stage of the microscope 12 and the objective 64 is focused thereon. Light from the objective 64 travels through the eyepiece 12 where it may be viewed by an observer. In addition, the optical converter module 14 includes a beam-splitting mirror 80 which carries off approximately 90% of the light from the objective 64 to other portions of the converter 14. The light is fed to a dual prism dichroic mirror 82 which reflects a portion of the light to the red filter 18. The remaining portion of the light is filtered by the dichroic mirror 82 and fed to the green filter 24. The dichroic mirror 82 selectively passes light having wavelengths greater than 500 nanometers to the filter 18 and having a wavelength of less than 500 nanometers to the filter 24. Thus, the dichroic mirror 82 acts as a first color filter before the light reaches the color filters 18 and 24.

When the light passes through the filter 18, the filter 18 preferentially blocks light from the green stained cell nuclei and provides a high contrast cell nuclei image to the camera 20. The camera 20 then generates an NTSC cell nuclei image signal which is fed to the image processor module 28. The image processor module 28 has an image processor 90 and an image processor 92. Each of the image processors 90 and 92 is a model AT428 from the Datacube Corporation. Similarly, the green filter 24, filter, provides a high contrast proliferation substance image to the camera 26. The

camera 26 then feeds the proliferation substance image signal to the image processor 92. Both of the image processors 90 and 92 contain analog to digital converters for converting the analog NTSC image signals to digitized arrays of pixels which are then stored within internal frame buffers. The internal frame buffers may be accessed via the system bus 34 under the control of the microprocessor 36.

The image of the cell sample viewed through the eyepiece 12 is of the type shown in FIG. 4 wherein a green cell nucleus 100, a green cell nucleus 102, a reddish-brown cell nucleus 104 having proliferation substance therein, a reddish-brown cell nucleus 106, and a reddish-brown and green cell nucleus 108 appear in an image field. As may best be seen in FIG. 5, the cell nuclei are shown therein as they would appear through the red filter 18, which causes all of the green cell nuclei to darken and appear prominently. As may best be seen in FIG. 6, the proliferation substance image of the cell nuclei of FIG. 4 is shown therein with the cell nuclei 100 and 102 being rendered substantially transparent or invisible by the effect of the green filter 24 which has its transmission peak at approximately the same wavelength as the transmission peak for the methyl green stain. The cell nuclei 104, 106 and 108 having the reddish-brown chromogen deposited therein which is an indicator for the proliferation substance appear clearly in high contrast.

The cell nuclei image of FIG. 5 is stored in the internal frame buffer of the image processor 90. The proliferation substance image of FIG. 6 stored in the internal frame buffer of the image processor 92. It may be appreciated that the pixel values for the images may be sliced using standard image processing techniques to increase the contrast between the cell nuclei and the backgrounds. That is, the areas of high optical density in FIG. 6 such as the cell nuclei 104, 106 and 108 may

be shown as being very dense and stored as high optical density pixels, while the background areas 110 may be stored in substantially zero optical density pixels in order to provide a clear threshold or difference between the two areas. This is particularly helpful when performing computations to determine the proliferation index, since the system can differentiate more easily between background and nuclei to be measured. This slicing technique acts as an additional amplifying step for the images.

Once the images have thus been acquired by the system, the user as may best be seen in FIG. 8, is interrogated as to whether the images are from a tissue section or a cell preparation. More particularly, after a starting step 120, the system 10 next displays an initial display screen 122 on the instruction monitor 52 and then interrogates the user in a step 124 as to whether a tissue section forms the basis for the image being processed. If the user provides a positive response to the system 10, control is transferred to a step 126 wherein a tissue section screen is displayed on the instruction monitor 52. If the response is negative, control is transferred to a step 128 where the user is questioned as to whether the cell sample is from a cell preparation. If the response is positive, control is transferred to a step 130 wherein a cell preparation processing and result screen of the type shown in FIG. 12 is displayed on the instruction monitor 52. In the event that neither of the selections is made, a step 132 transfer control to a HELP screen 134.

Referring back to the step 126, it may be appreciated that the screen of FIG. 11 is displayed during the step 126. The screen provides a menu of functions at the right-hand side which are of the type well known to users of automated cell analysis equipment. In particular, the user may select a nuclear

threshold function wherein the user may specify the threshold optical density or pixel value at which the system 10 determines for purposes of computation that a particular pixel value is indicative of the presence of a portion of a cell nucleus at that point. Furthermore, an antibody threshold may similarly be set wherein the optical density of the image of FIG. 6 is measured and a threshold is set indicative of the presence or absence of antibody at a particular pixel address. In addition, the user, once having set the thresholds, may then display outlines or shaded areas of the cell nuclei and the antibodies in a display nuc-anti masking function. Once the user does this, control is transferred to a tissue section analysis step 140 which may be seen in more detail in FIG. 9.

A 620 nanometer cell nuclei image of the type is received by the camera 20 in a step 150. The analogy image signal is digitized in a step 152 and a threshold value for pixels indicating the presence of the cell nuclei is selected in a step 154. Once the threshold has been selected, pixels having a value less than the threshold have their values set to a pre-selected background level while the pixels having values over leaving a high contrast pixel array for further processing. The pixel array is transferred to the computer system 32 where the number of pixels having values exceeding the selected nuclear threshold value is counted to provide a cell nuclei amount or count which will be used as a proliferation index denominator in later processing.

Similarly, the proliferation substance image of the type shown in FIG. 6 is received by the camera 26 in a step 160. The proliferation substance image is digitized by the image processor 92 in a step 162. An antibody threshold which has been selected by the user reduces the background of the proliferation substance

image to zero and effectively isolates the pixels representative of the proliferation substance antibody in a step 164. The isolated pixels, that is those pixels having a value greater than the preselected antibody threshold, are then counted by the system 32 and a pixel count number 162 is provided in the step 166.

Thus, it may be appreciated that steps 150 through 156 effectively measure the area of the image field of FIG. 5 wherein cell nuclei are found. The steps 160 through 166 effectively measure the area of the proliferation substance in the image field of FIG. 6. The computer 32 in a step 168 then divides the proliferation substance by the area of the cell nuclei and generates a quotient which is equal to the proliferation index. The proliferation index is then displayed on the tissue section screen as a percentage number. In addition, the total nuclear area as computed in steps 150 through 156 is also displayed.

In the event that the user has indicated to the system in the step 128 that a cell preparation is being analyzed, control is transferred to step 130 which may be seen in more detail in a step 170 as shown in FIG. 10. In a step 200, the cell nuclei image of FIG. 5 is received by the camera 20. The cell nuclei image is digitized in a step 202. The digitized cell nuclei image is then analyzed in a step 204 to determine, using neighborhood labelling, what objects are to be considered by the system 10 to be cell nuclei and what objects are not. The objects to be considered to be cell nuclei are indicated by being surrounded by boxes as displayed on the image monitor 30. In a step 206, if two or more of the objects are in contact with each other, the operator is given the opportunity to have the system draw a line of demarkation in between then or to manually separate the images himself.

In a step 210, a threshold value is then applied to the pixel arrays in a step 208 to amplify the differences among pixels by slicing, as was done in steps 154 and 164 previously. Similarly, in a step 212, the proliferation substance image of FIG. 6 is received by the camera 26. The proliferation substance image is digitized in the step 214 and is isolated in a step 216. The cell nuclei and proliferation substance pixel arrays are then combined in a step 218 and displayed on the image monitor 30. The cell nuclei are counted by the computer 32. Likewise, the cell nuclei having proliferation substance are also counted by the computer 32. The number of proliferation substance nuclei is then divided by the total number of cell nuclei to produce a proliferation index for the cell preparation sample. The proliferation index is then displayed on the cell preparation screen of FIG. 12.

It may thus be appreciated that the tissue section feature of FIG. 9 allows the proliferation index for a tissue section sample to be easily and rapidly computed using stereological principals which are standard in the field of microscropy. When tissue sections are not used and stereological principals do not apply, the cells may be counted by using the cell principal preparation technique.

Furthermore, the system provides considerable amplification for determination of the proliferation index. The initial amplification takes place when the proliferation substance is identified with the chromogen and the cell nuclei are stained with the counterstain. A second amplification takes place when the cell nuclei and proliferation substance images are formed by filtering the light through the optical filters 18 and 24. Further amplification takes place when the threshold values for the proliferation substance and the cell nuclei are set providing high contrast images and high gain digital arrays for further processing.

While there has been illustrated and described a particular embodiment of the present invention, it will be appreciated that numerous changes and modifications will occur to those skilled in the art, and it is intended in the appended claims to cover all of those changes and modifications which fall within the true spirit and scope of the present invention.

WHAT IS CLAIMED IS:

1. An apparatus for determining a proliferation index of a cell sample, comprising;

proliferation substance sensing means for sensing portions of a cell sample having a proliferation substance and producing a proliferation substance signal in response thereto;

cell nuclei sensing means for sensing portions of a cell sample having cell nuclei and producing a cell nuclei signal in response thereto;

proliferation substance determining means coupled to the proliferation substance sensing means and receiving the proliferation substance signal, for determining an amount of the proliferation substance from the proliferation substance signal and producing a proliferation substance amount signal;

cell nuclei determining means coupled to the cell nuclei sensing means and receiving the cell nuclei signal, for determining an amount of cell nuclei and producing a cell nuclei amount signal in response thereto; and

proliferation index determining means coupled to the proliferation substance determining means and receiving the proliferation substance amount signal and coupled to the cell nuclei determining means and receiving the cell nuclei amount signal therefrom, for determining a proliferation index from the proliferation substance amount signal and the cell nuclei amount signal.

2. An apparatus for determining a proliferation index of a cell sample as defined in claim 1, wherein the proliferation substance sensing means further comprises a proliferation substance image enhancing optical filter

which blocks a transmission peak of a chromogen associated with the proliferation substance but transmits a transmission peak of a counterstain associated with the cell nuclei.

3. An apparatus for determining a proliferation index of a cell sample as defined in claim 1, wherein the cell nuclei sensing means further comprises a cell nuclei image enhancing optical filter which blocks a transmission peak of a counterstain associated with the cell nuclei but transmits a transmission peak of a chromogen associated with the proliferation substance.

4. An apparatus for determining a proliferation index of a cell sample as defined in claim 3, wherein the proliferation substance sensing means further comprises a proliferation substance image enhancing optical filter which blocks a transmission peak of a chromogen associated with the proliferation substance but transmits a transmission peak of a counterstain associated with the cell nuclei.

5. An apparatus for determining a proliferation index of a cell sample as defined in claim 4, wherein the proliferation substance sensing means further comprises means for storing a digitized proliferation substance substance image array.

6. An apparatus for determining a proliferation index of a cell sample as defined in claim 1, wherein the proliferation substance determining means further comprises means for determining an image area occupied by the proliferation substance and the proliferation substance amount signal is indicative of the image area occupied by the proliferation substance.

7. An apparatus for determining a proliferation index of a cell sample as defined in claim 6, wherein the cell nuclei determining means further comprises means for determining an image area occupied by the cell nuclei and the cell nuclei amount signal is indicative of the image area occupied by the cell nuclei.

8. An apparatus for determining a proliferation index of a cell sample as defined in claim 1, wherein the proliferation substance determining means further comprises means for determining a number of cell nuclei in an image field having proliferation substance therein and the proliferation substance amount signal is indicative of the number of cell nuclei having proliferation substance.

9. An apparatus for determining a proliferation index of a cell sample as defined in claim 8, wherein the cell nuclei determining means further comprises means for determining a number of cell nuclei in the image field and the cell nuclei amount signal is indicative of the cell nuclei in the image field.

10. A method for determining a proliferation index of a cell sample, comprising the steps of:

staining a plurality of cell objects with a monoclonal antibody-stain complex specific for a proliferation substance;

staining the plurality of cell objects with a counterstain;

sensing an image of the plurality of cell objects;

enhancing the image of the stained cell objects to produce an enhanced image; and

measuring automatically an amount of the proliferation substance in the stained cell objects from the enhanced image.

11. A method for determining a proliferation index of a cell sample as defined in claim 10, wherein the proliferation substance comprises an antigen for which Ki-67 is an antibody.

12. A method for determining a proliferation index of a cell sample as defined in claim 10, wherein the proliferation substance comprises 5-bromodeoxyuridine.

13. A method for determining a proliferation index of a cell sample as defined in claim 10, wherein the proliferation substance comprises a protein.

14. A method for determining a proliferation index of a cell sample as defined in claim 10, wherein the proliferation substance comprises cyclin.

15. A method for determining a proliferation index of a cell sample as defined in claim 10, wherein the monoclonal antibody-stain complex comprises peroxidase.

16. A method for determining a proliferation index of a cell sample as defined in claim 15, wherein the monoclonal antibody-stain complex further comprises anti-peroxidase.

17. A method for determining a proliferation index of a cell sample as defined in claim 16, wherein the monoclonal antibody-stain complex further comprises diaminobenzidine

18. A system for performing an image enhancement operation on an image of a plurality cell objects, comprising:

sensor means for sensing an image of a plurality of cell objects and producing an image signal in response thereto;

mask control means for receiving a mask control signal from a user, said mask control signal having a first state and a second state;

mask generator means for generating automatically a mask for each cell object image when said cell object image has a characteristic within a preselected range and said mask control signal is in said second state;

cell object tag means for producing a user perceivable indication for each cell object within a specified image field when said mask control signal is in said first state; and

means for suspending the mask generating function until the means for receiving the mask signal receives an enable signal for the masking operation for said cell object identified by the cell object tag means.

19. A system for analyzing a predetermined characteristic of a plurality of cells in a tissue section or in a cell preparation, comprising:

means for examining a plurality of cells in a cell sample and generating an image signal representative of the plurality of cell images displayed in an image field;

storage means, coupled to the optical means for storing the image signal;

mode select means for determining whether the image signal is to be evaluated on the basis of the cell images originating from a tissue section or from a cell preparation;

cell analysis means, coupled to the storage means and to the mode select means for quantitatively analyzing the cell images for a predetermined characteristic; and

output means for outputting a result of the quantitative analysis of the predetermined characteristic.

20. A system for assaying a quantity of a proliferation substance in a cell sample, comprising:

optical means for examining a plurality of cells in a cell sample and generating an image signal representative of the plurality of cell images displayed in an image field;

storage means, coupled to the optical means for storing the image signal;

assay mode select means for interrogating a user as to whether the image signal is to be evaluated on the basis of the cell images originating from a tissue section or a cell preparation and generating a tissue section option signal or a cell preparation option signal in response thereto;

tissue select means, coupled to the storage means and to the assay mode select means for determining an amount of a proliferation substance in the cell sample on an area basis and generating an assay thereof, said tissue select means means being activated in response to a tissue option being selected;

cell preparation means, coupled to the assay mode select input means and to the storage means for determining an amount of the proliferation substance in the cell sample on the basis of a tally of a number of cell images stored which show a positive response for the proliferation substance and generating an assay thereof; and

output means for outputting the results of the assay to the user.

21. A system for performing an image enhancement operation on an image of a cell sample, comprising:

image analysis means having a sensor means for sensing an image of a plurality of cells and generating an image signal in response thereto;

means for defining a shared cell image resulting from a pair of cell images sharing a contiguous portion;

means for defining a shared cell boundary for segregating the shared cell image into a pair of separate cell images for further processing; and

means for displaying the shared cell boundary.

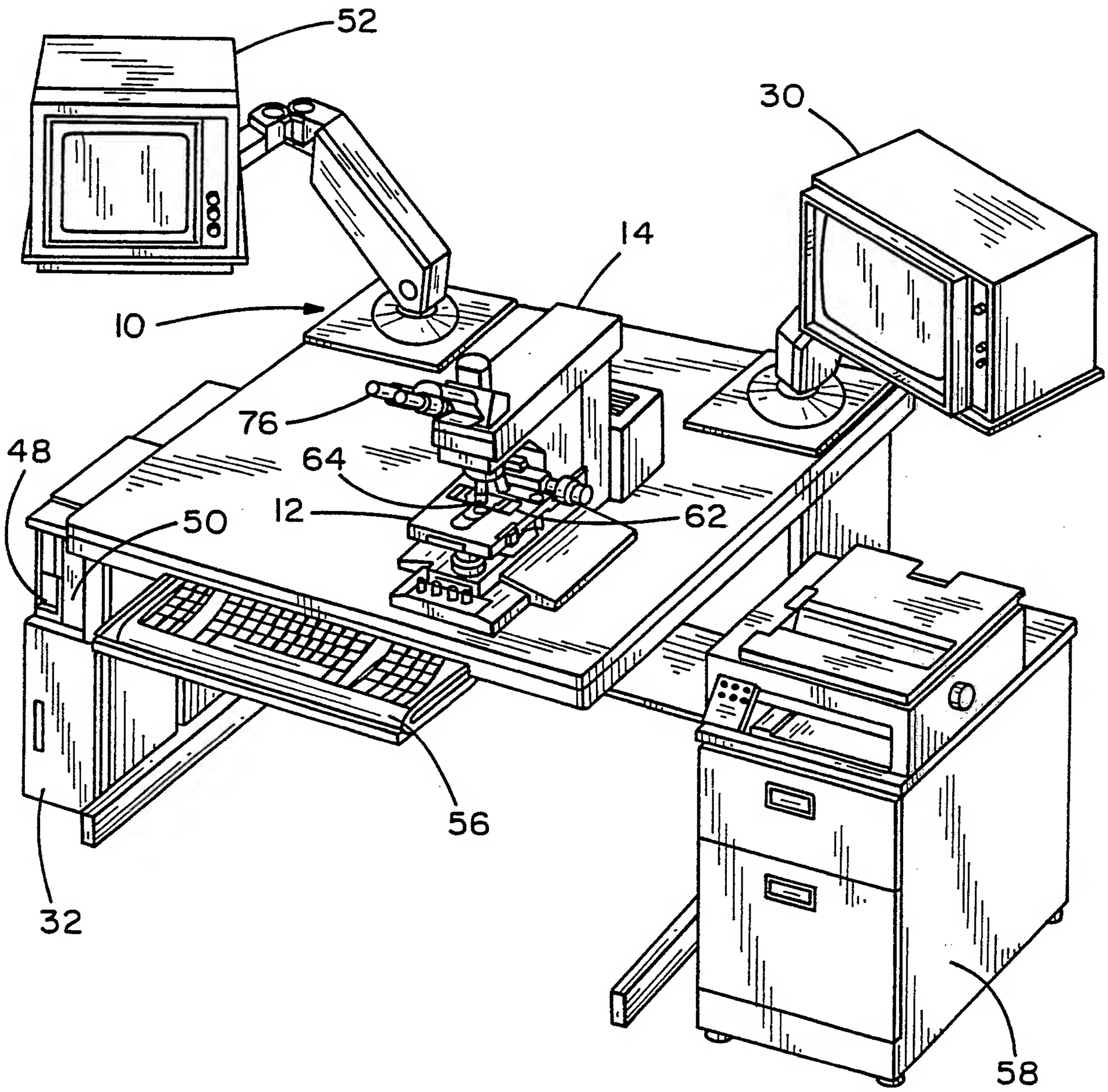


FIG. 1

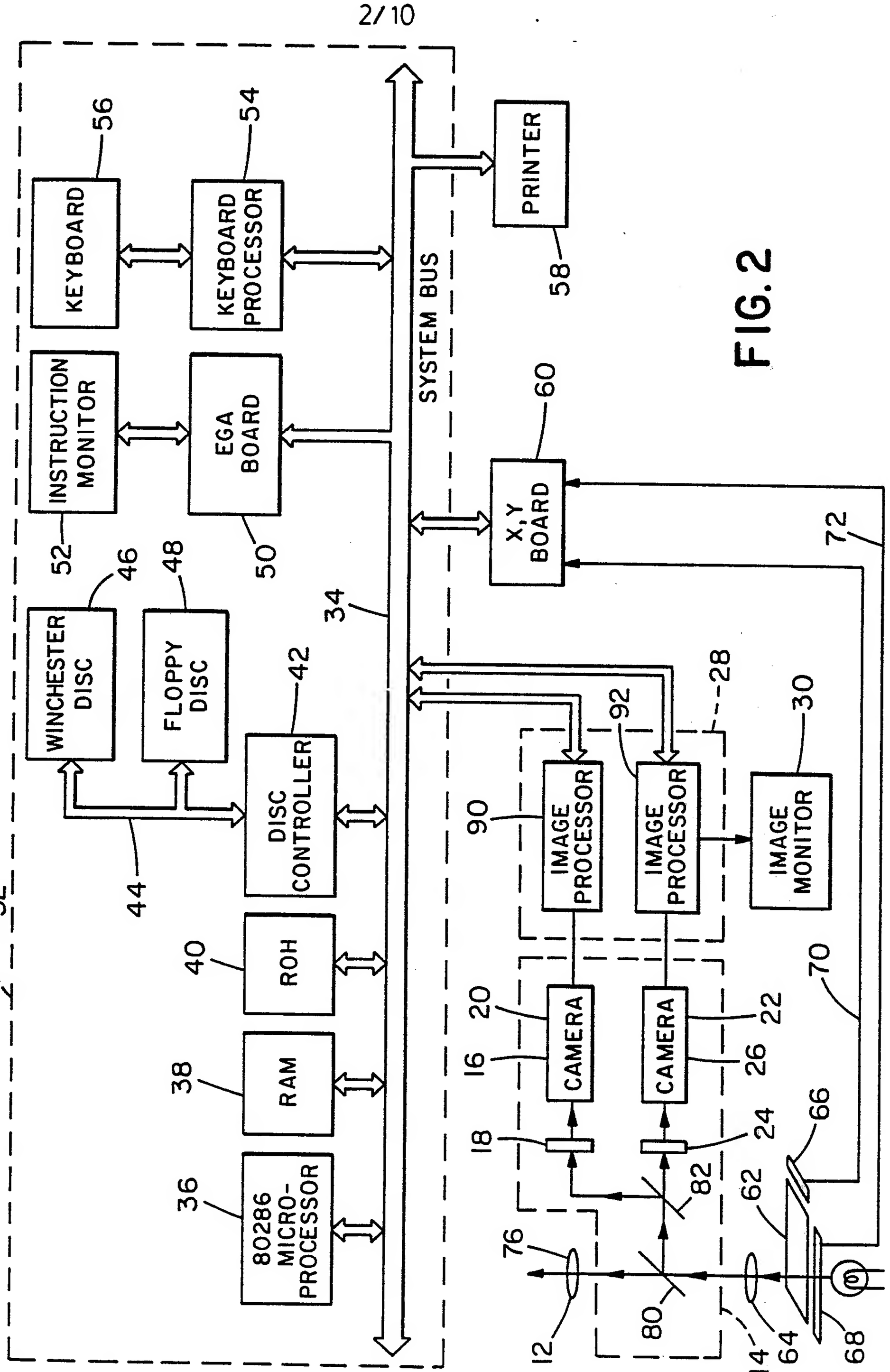

KEYBOARD
56
KEYBOARD PROCESSOR
54
PRINTER
58
SYSTEM BUS
INSTRUCTION MONITOR
52
EGA BOARD
50
X,Y BOARD
60
72
34
WINCHESTER DISC
46
FLOPPY DISC
48
DISC CONTROLLER
42
44
32
IMAGE PROCESSOR
90
IMAGE PROCESSOR
92
28
IMAGE MONITOR
30
ROH
40
RAM
38
80286 MICRO-PROCESSOR
36
CAMERA
20
16
CAMERA
22
26
70
18
24
66
82
62
76
80
12
14
64
68

FIG. 2

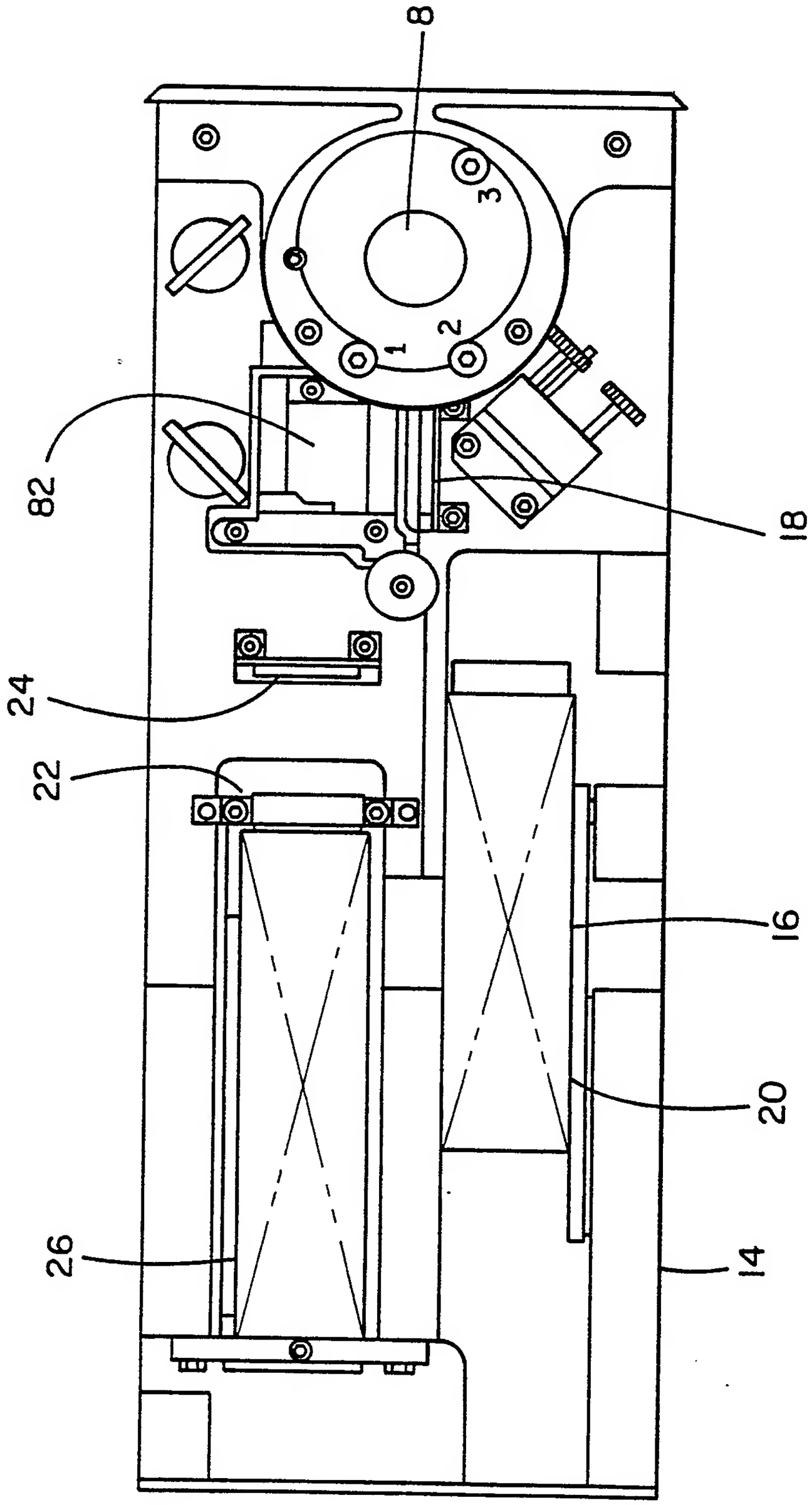
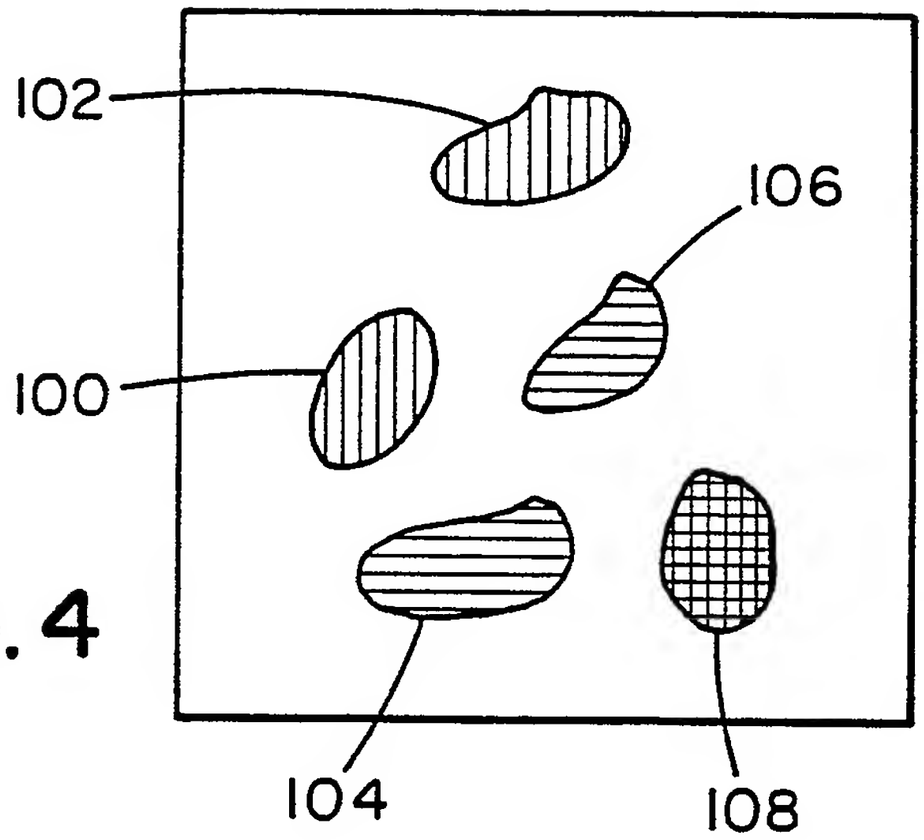


FIG.3



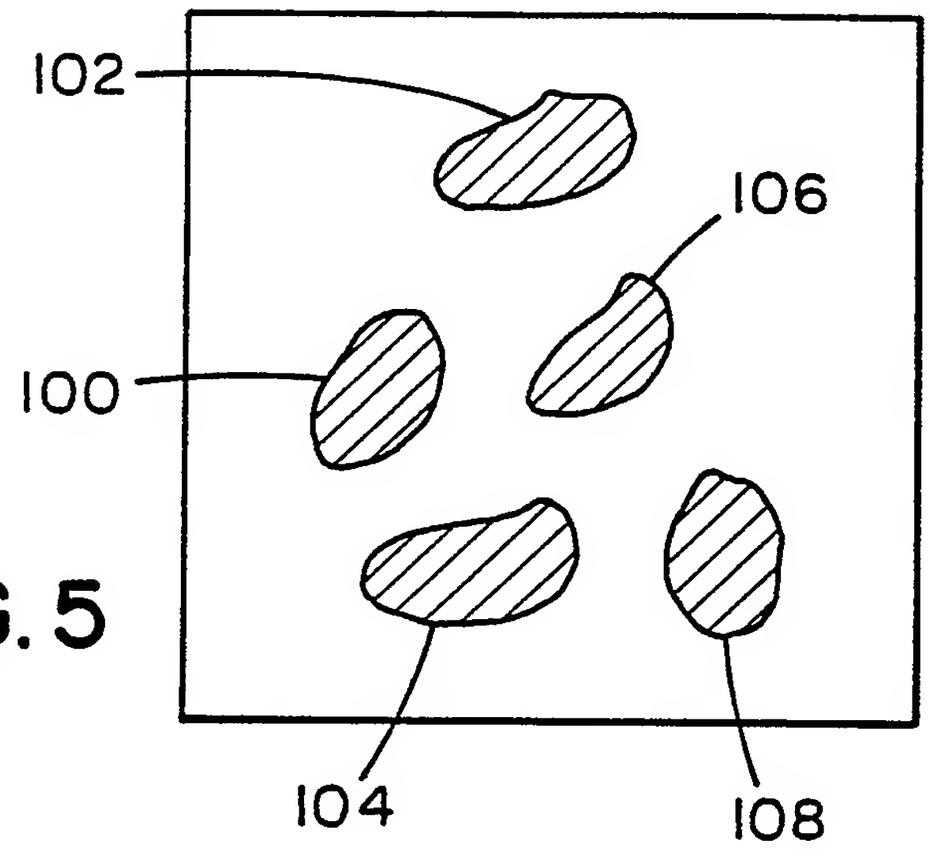
ORIGINAL IMAGE SEEN THOUGH WHITE LIGHT

|||| GREEN

BROWN

GREEN AND BROWN

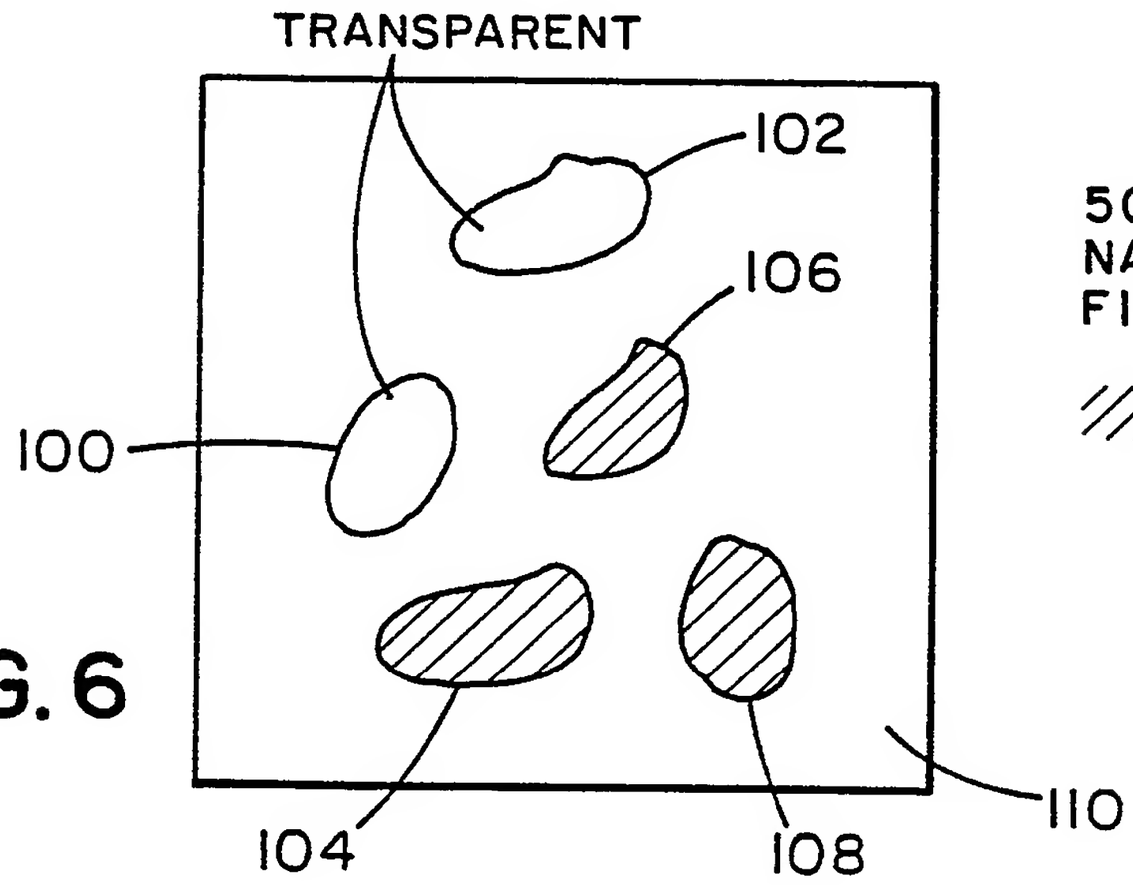
FIG. 4



620 NM (RED) NARROW BANDPASS FILTERED IMAGE

/// GREY SCALE OR OPTICAL DENSITY

FIG. 5



500 NM (GREEN) NARROW BANDPASS FILTERED IMAGE

/// GREY SCALE OR OPTICAL DENSITY

FIG. 6

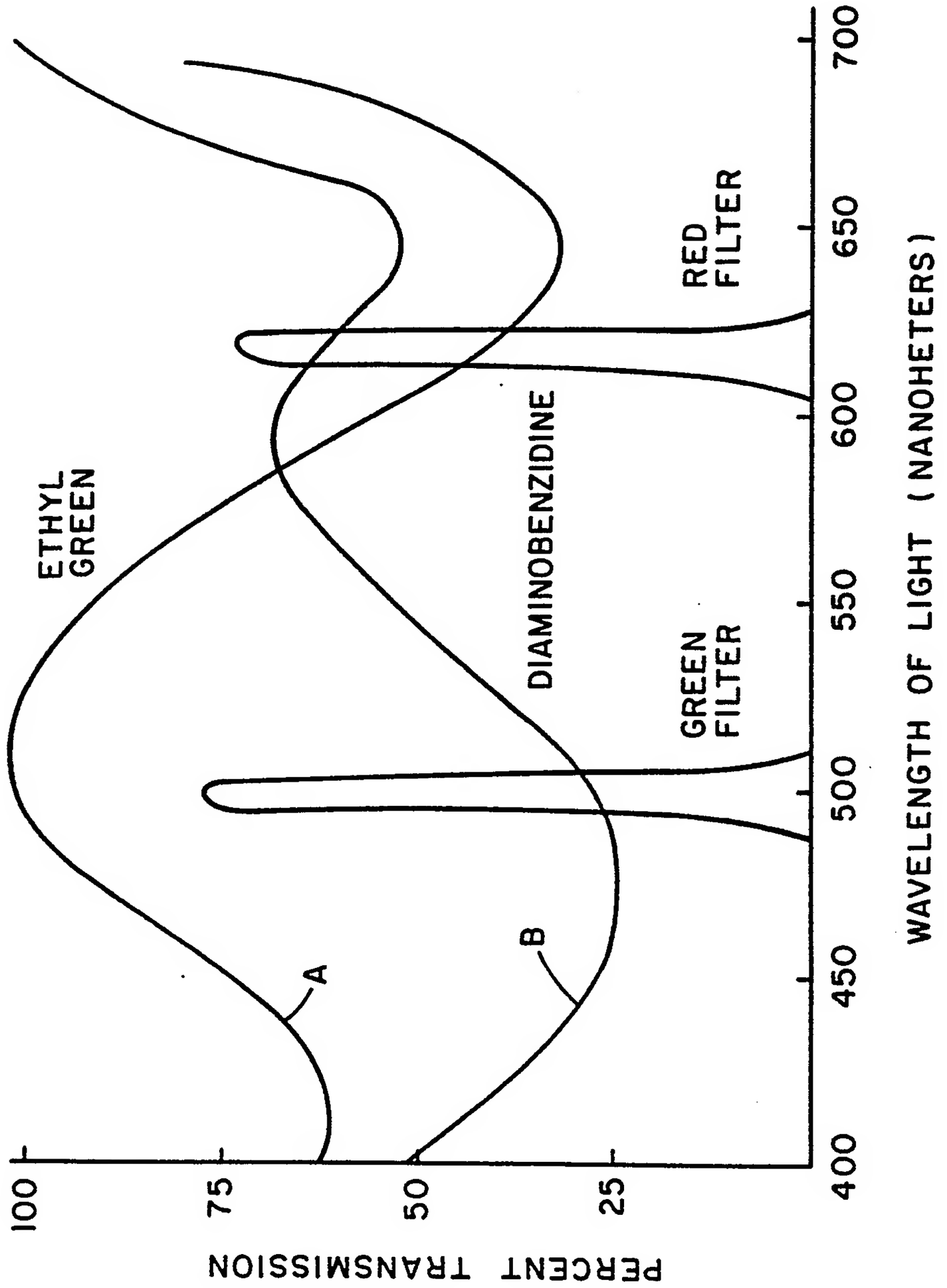

ETHYL GREEN
DIAMINOBENZIDINE
GREEN FILTER
RED FILTER
A
B
100
75
50
25
PERCENT TRANSMISSION
400
450
500
550
600
650
700
WAVELENGTH OF LIGHT (NANOHETER

FIG. 7

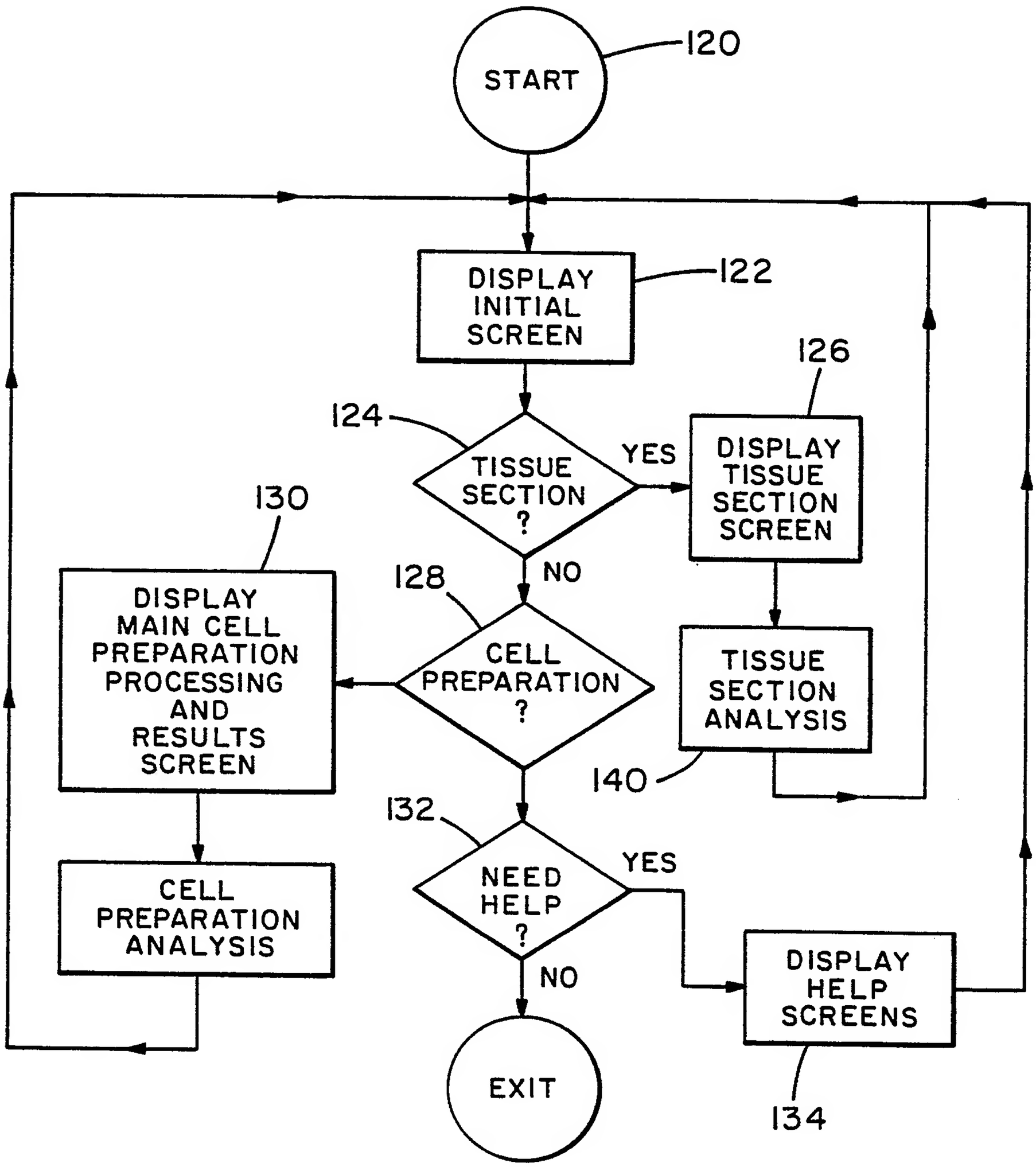

START
120
DISPLAY INITIAL SCREEN
122
TISSUE SECTION ?
124
YES
DISPLAY TISSUE SECTION SCREEN
126
NO
CELL PREPARATION ?
128
DISPLAY MAIN CELL PREPARATION PROCESSING AND RESULTS SCREEN
130
CELL PREPARATION ANALYSIS
TISSUE SECTION ANALYSIS
140
NEED HELP ?
132
YES
NO
DISPLAY HELP SCREENS
134
EXIT

FIG. 8

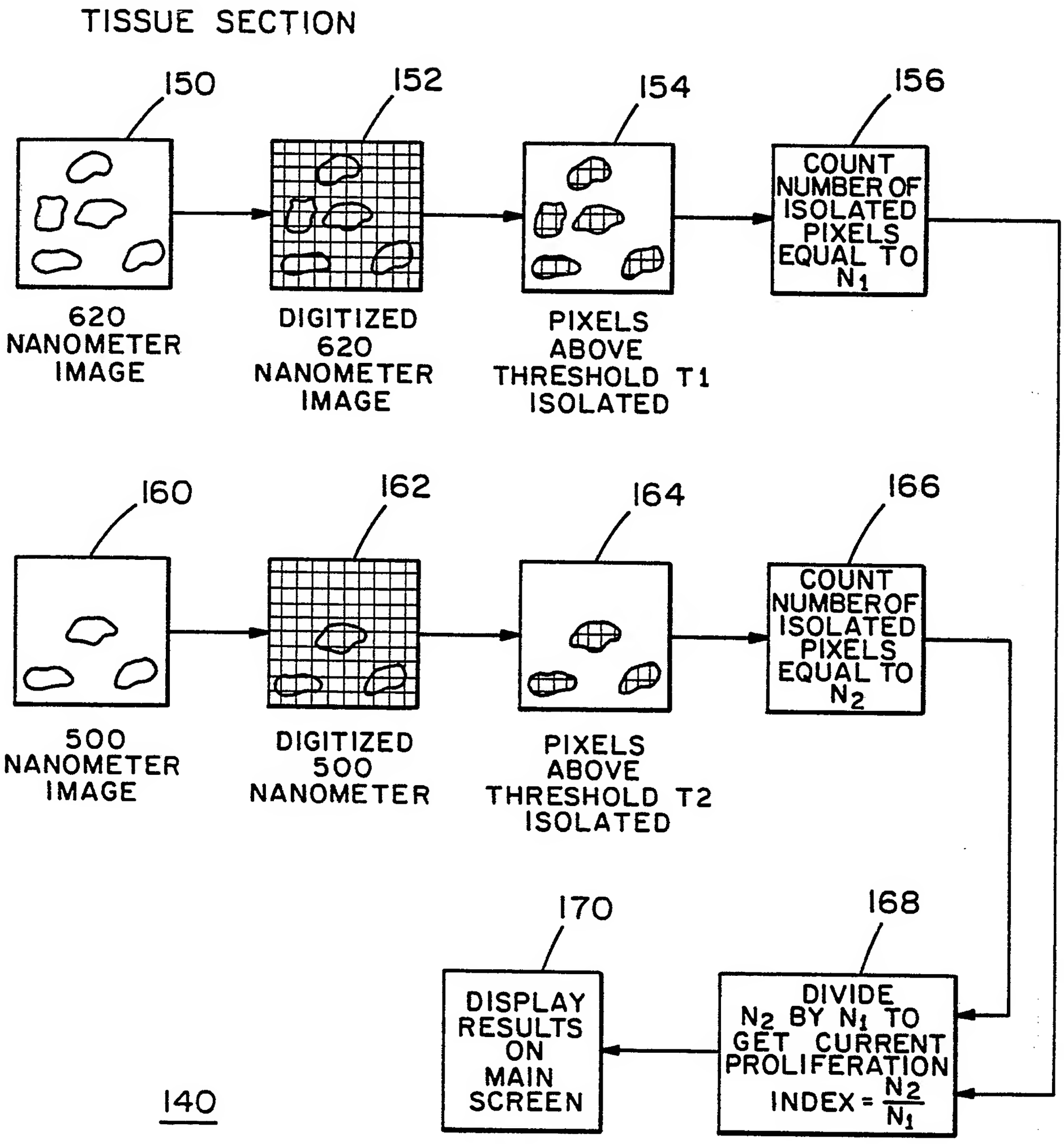

TISSUE SECTION
150
620 NANOMETER IMAGE
152
DIGITIZED 620 NANOMETER IMAGE
154
PIXELS ABOVE THRESHOLD T1 ISOLATED
156
COUNT NUMBER OF ISOLATED PIXELS EQUAL TO N_1
160
500 NANOMETER IMAGE
162
DIGITIZED 500 NANOMETER
164
PIXELS ABOVE THRESHOLD T2 ISOLATED
166
COUNT NUMBER OF ISOLATED PIXELS EQUAL TO N_2
170
DISPLAY RESULTS ON MAIN SCREEN
168
DIVIDE N_2 BY N_1 TO GET CURRENT PROLIFERATION INDEX = $\frac{N_2}{N_1}$
140

FIG. 9

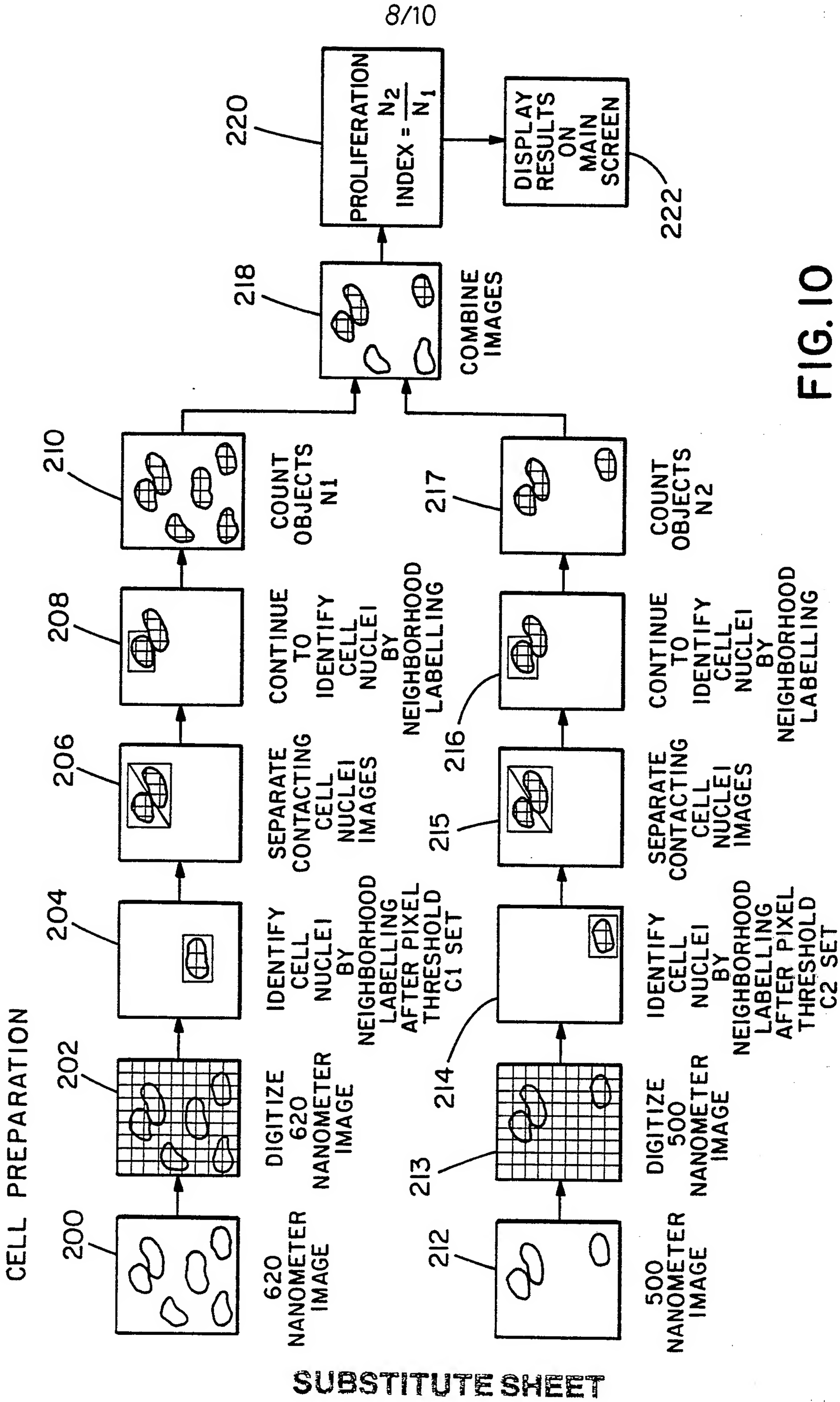

CELL PREPARATION
200
620 NANOMETER IMAGE
DIGITIZE 620 NANOMETER IMAGE
202
204
IDENTIFY CELL NUCLEI BY NEIGHBORHOOD LABELLING AFTER PIXEL THRESHOLD C1 SET
206
SEPARATE CONTACTING CELL NUCLEI IMAGES
208
CONTINUE TO IDENTIFY CELL NUCLEI BY NEIGHBORHOOD LABELLING
210
COUNT OBJECTS N1
212
500 NANOMETER IMAGE
DIGITIZE 500 NANOMETER IMAGE
213
214
IDENTIFY CELL NUCLEI BY NEIGHBORHOOD LABELLING AFTER PIXEL THRESHOLD C2 SET
215
SEPARATE CONTACTING CELL NUCLEI IMAGES
216
CONTINUE TO IDENTIFY CELL NUCLEI BY NEIGHBORHOOD LABELLING
217
COUNT OBJECTS N2
218
COMBINE IMAGES
220
PROLIFERATION INDEX = $\frac{N_2}{N_1}$
222
DISPLAY RESULTS ON MAIN SCREEN

FIG. 10

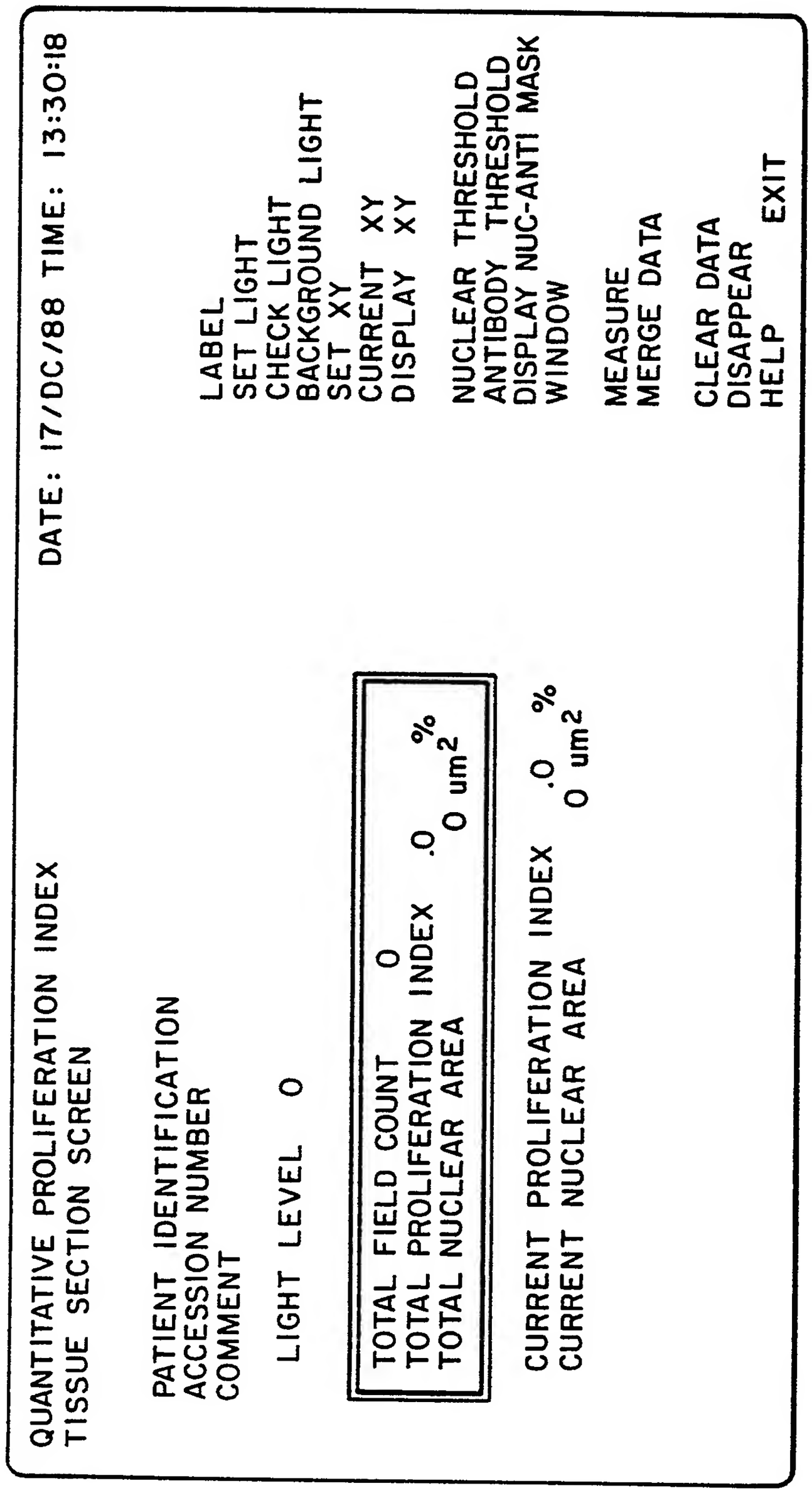

QUANTITATIVE PROLIFERATION INDEX
TISSUE SECTION SCREEN
DATE: 17/DC/88 TIME: 13:30:18
PATIENT IDENTIFICATION
ACCESSION NUMBER
COMMENT
LIGHT LEVEL 0
TOTAL FIELD COUNT 0
TOTAL PROLIFERATION INDEX .0 %
TOTAL NUCLEAR AREA 0 um2
CURRENT PROLIFERATION INDEX .0 %
CURRENT NUCLEAR AREA 0 um2
LABEL
SET LIGHT
CHECK LIGHT
BACKGROUND LIGHT
SET XY
CURRENT XY
DISPLAY XY
NUCLEAR THRESHOLD
ANTIBODY THRESHOLD
DISPLAY NUC-ANTI MASK
WINDOW
MEASURE
MERGE DATA
CLEAR DATA
DISAPPEAR
HELP
EXIT

FIG. II

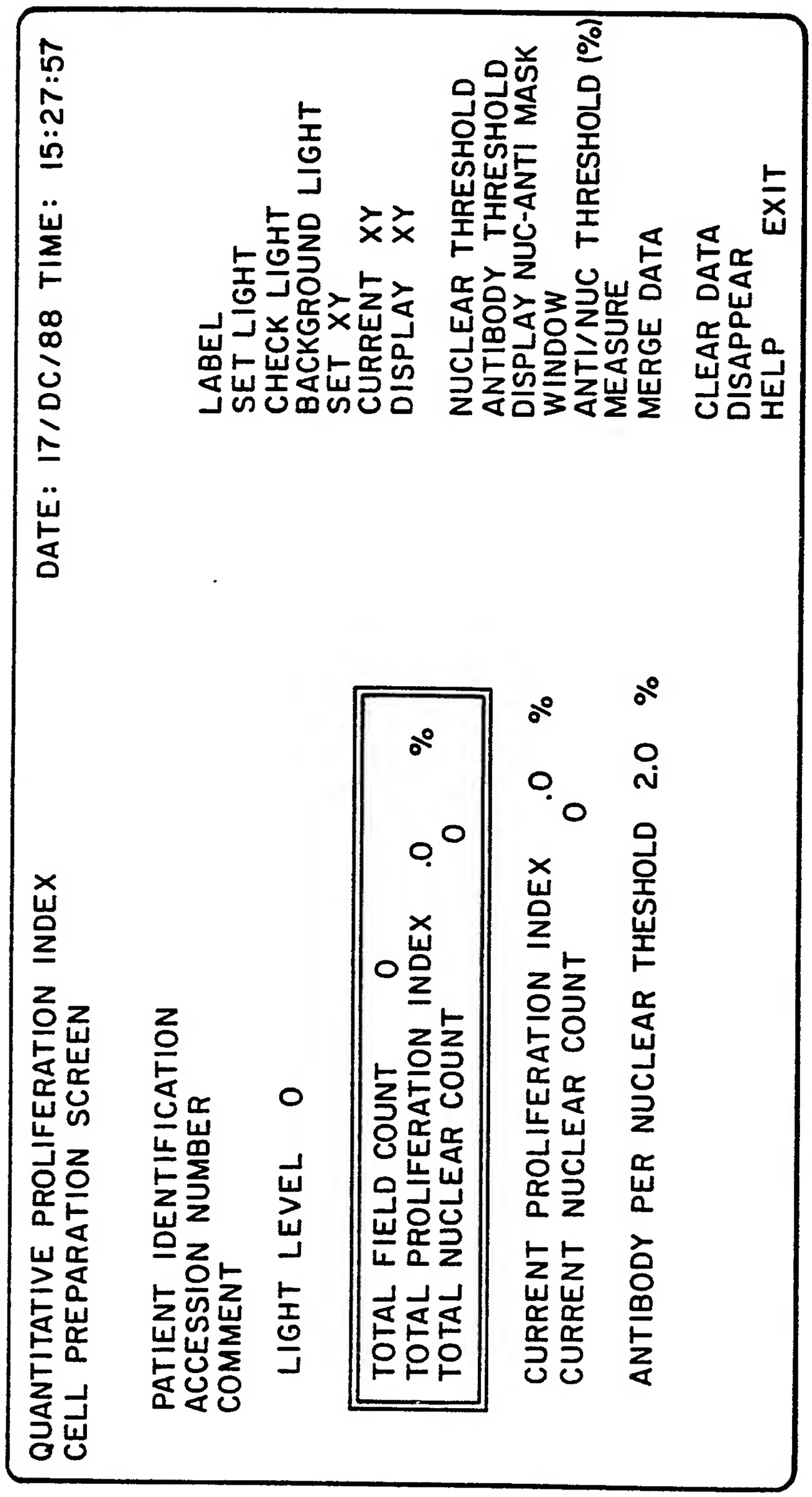

QUANTITATIVE PROLIFERATION INDEX
CELL PREPARATION SCREEN
DATE: 17/DC/88 TIME: 15:27:57
PATIENT IDENTIFICATION
ACCESSION NUMBER
COMMENT
LIGHT LEVEL 0
TOTAL FIELD COUNT 0
TOTAL PROLIFERATION INDEX .0 %
TOTAL NUCLEAR COUNT 0
CURRENT PROLIFERATION INDEX .0 %
CURRENT NUCLEAR COUNT 0
ANTIBODY PER NUCLEAR THESHOLD 2.0 %
LABEL
SET LIGHT
CHECK LIGHT
BACKGROUND LIGHT
SET XY
CURRENT XY
DISPLAY XY
NUCLEAR THRESHOLD
ANTIBODY THRESHOLD
DISPLAY NUC-ANTI MASK
WINDOW
ANTI/NUC THRESHOLD (%)
MEASURE
MERGE DATA
CLEAR DATA
DISAPPEAR
HELP
EXIT

FIG.12

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/00999

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): G06K 9/34

US CL.: 382/6; 356/39; 364/416; 128/653, 665

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	382/6 364/416 356/39 128/653, 665

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	US,A, 4,741,043 (BACUS) 26 April 1988 See figures 1-2 and abstract	
A	US,A, 4,199,748 (BACUS) 22 April 1980 See abstract	
A	US,S, 4,404,683 (KOBAYASHI et al) 13 September 1983 See the abstract	
A	US,A, 4,125,828 (RESNICK et al) 14 November 1978 See abstract	

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

14 May 1990

Date of Mailing of this International Search Report

24 JUL 1990

International Searching Authority

ISA/US

Signature of Authorized Officer

Michael Razavi